REMARKS

Entry of the foregoing and reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested in light of the remarks which follow.

Applicants note with appreciation that claims 1-14, 19-23, and 26 are allowed. Claims 4 and 20 are amended herein to recite the full name of the claims HGK1 protein. Claims 15-16 and 24-25 are canceled herein without prejudice or disclaimere thereto. Applicants reserve the right to file at least one continuation application directed to any subject matter canceled herein.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 15-16, 24-25 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Office argues that claims 15-16 and 24-25 are directed to disease conditions distinct from each other, and that the specification does not provide guidance for using the claimed antibody with these diseases.

In the interest of expediting prosecution and without acquiescing in the rejection, claims 15-16 and 24-25 are canceled herein without prejudice or disclaimer thereto. Thus, this rejection is moot.

Claim Rejections Under 35 U.S.C. § 112, second paragraph

Claims 4 and 20 stand rejected under 35 U.S.C. § 112, second paragraph, as purportedly indefinite, for the recitation of "HGKI". The Office requests that the claims be amended to recite the full name of the claimed HGK protein. Thus, claims

4 and 20 are amended herein to recite "human glandular kallikrein 1 (HGK1)". In support, Applicants refer to page 7, lines 26-31 of the specification as reciting "HGK1": as well as a printout of the Information Hyperlinked Over Proteins (iHOP) (Exhibit A) protein website showing that HGK1 is an abbreviation for the human glandular kallikrein 1 protein. In light of this amendment, Applicants request that this rejection be withdrawn.

CONCLUSION

From the foregoing, further and favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

In the event that there are any questions concerning this amendment or the application in general, the Examiner is respectfully requested to telephone the undersigned so that prosecution of the application may be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

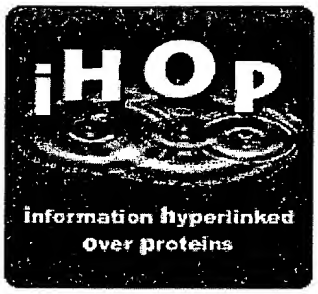
Date: April 11, 2005

By: _____
Deborah H. Yellin
Registration No. 45,904

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

VA 714900.1

EXHIBIT A



Symbol	Name	Synonyms	Organism
KLK2	Kallikrein 2 precursor	Glandular kallikrein-1, hGK-1, kallikrein 2, prostatic, Tissue kallikrein 2	Homo sapiens

UniProt	P20151, Q15946, Q9UJZ9
OMIM	147960
NCBI Gene	3817
NCBI RefSeq	NP_005542
NCBI Accession	AAA74454, AAD13816, AAD13817

Homologues of KLK2 ... new

Definitions for KLK2 ...

Enhanced PubMed/Google query ... new

WARNING: Please keep in mind that gene detection is done automatically and can exhibit a certain error. Read more.

Search Gene

Show overview
Find in this Page

Filter and options
Gene Model

Developer's Zone

Help

Find in this Page

Three family members have been characterized in the human: glandular kallikrein (KLK1) and two genes expressed in the prostate, KLK2 or hGK1 and KLK3 or prostate specific antigen (PSA).

Prostate-specific antigen (PSA) and human glandular kallikrein (hGK-1) together constitute a subfamily of serine proteases exclusively produced in the human prostate.

Here, we describe the precise mapping and localization of the prostase/KLK-L1 gene between the known genes KLK2 (human glandular kallikrein) and zyme (also known as protease M/neurosin).

3. An oligonucleotide probe complementary to human glandular kallikrein-1 coding region (amino acids 161-167) detected a single DNA fragment after digestion with Bam H1, Hind III or Pst 1.

A range of luciferase reporter vectors was constructed, incorporating 5'-flanking sequences from the prostate-specific antigen (PSA), human glandular kallikrein 2 (hKLK2), and cytomegalovirus (CMV) promoters for expression control.

PSA and human glandular kallikrein (hK2, previously called hGK-1) share extensive homology and are both produced in the prostate under androgen control.

Prostate-specific antigen (PSA) and human glandular kallikrein 1 (hGK-1) are structurally similar products of the human glandular kallikrein gene locus on chromosome 19 that become selectively expressed by human prostate tissue.

The human prostate-specific kallikreins, human glandular kallikrein-1 (hKLK2) and prostate-specific antigen (hKLK3), have been shown to be regulated by androgens.

On Southern blot analysis with gene-specific oligonucleotide probes, we have detected expression of the three human KLK genes--KLK1 (kallikrein), KLK2 (or hGK1) and KLK3 (PSA).

The human kallikrein gene family is composed of three members:

Concept & Implementation
by Robert Hoffmann

tissue kallikrein (KLK1), prostate-specific antigen (PA or APS), and human glandular kallikrein-1 (hGK-1 or KLK2).

The human kallikrein gene family is localized on chromosome 19q13.3-q13.4 and currently includes three members: KLK1 or pancreatic/renal kallikrein, KLK2 or human glandular kallikrein and KLK3 or prostate-specific antigen (PSA).

The gene encoding human glandular kallikrein (KLK2) was expressed in Escherichia coli, and the corresponding protein (hK2) was produced by fermentation.

The traditional human kallikrein gene family consists of three genes, namely KLK1 [encoding human kallikrein 1 (hK1) or pancreatic/renal kallikrein], KLK2 (encoding hK2, previously known as human glandular kallikrein 1) and KLK3 [encoding hK3 or prostate-specific antigen (PSA)].

The human prostate-specific antigen (PSA) and kallikrein 2 (KLK2) genes are regulated by the androgen receptor (AR).

1. Humans have three kallikrein genes: hGK-1, hRKALL (kinin-generating) and a gene encoding prostate specific antigen (PSA).

Unusual alternative splicing within the human kallikrein genes KLK2 and KLK3 gives rise to novel prostate-specific proteins.

The human kallikrein gene cluster, located in the chromosome band 19q13, contains several tissue-specific serine protease genes including the prostate-specific KLK2, KLK3 and prostase genes.

The human tissue kallikrein (KLK) family of serine proteases, which is important in post-translational processing events, currently consists of just three genes-tissue kallikrein (KLK1), KLK2, and prostate-specific antigen (PSA) (KLK3)-clustered at chromosome 19q13.

Prostate-specific antigen (PSA) and human kallikrein 2 are closely related products of the human kallikrein genes KLK3 and KLK2, respectively.

KLK5 is a newly discovered human kallikrein gene which shares a high degree of homology and is located adjacent to KLK2 and KLK3 genes on chromosome 19q13.

top

To determine whether the androgen induction of these genes is transcriptionally regulated via an androgen response element, an hKLK2 promoter DNA fragment was linked to a promoterless chloramphenicol acetyltransferase (CAT) reporter gene and cotransfected with an androgen receptor expression vector in an androgen receptor-less human prostate cell line, PC-3.

Mouse orthologs to all human kallikrein genes, except for KLK2 and KLK3 genes, have now been identified.

In situ hybridization revealed that, in prostate, KLK14 is expressed by both benign and malignant glandular epithelial cells, thus exhibiting an expression pattern similar to that of two other prostatic kallikreins, KLK2 and KLK3, which encode K2 and prostate-specific antigen, respectively.

We examined previously the steroid hormone regulation of 2 known androgen-regulated kallikreins, KLK3 (encoding PSA) and KLK2 (encoding human kallikrein 2 or hK2) in BT-474, T-47D, ZR75-1, MCF-7, MFM-223 and BT-20 human breast cancer cells and found that they were differentially regulated, with the cells showing variable responses to androgen.

These results indicate that a Fos-containing protein complex distinct from AP-1 **binds** upstream of the AREs in the PSA and KLK2 promoters, interacts with the AR and may participate in regulation of these two androgen-responsive genes.

The identity of KLK1 and KLK2 was confirmed by sequencing the PCR products.

Stimulation of PSA RNA is about 5-fold, whereas **hGK-1** stimulation is less pronounced.

A time course study showed that both **hKLK2** and c-myc mRNAs were repressed by TPA as early as four hours.

RNA analysis revealed that both genes are expressed in LNCaP cells with PSA basal levels being 2-fold higher than **hGK-1** levels.

A binding motif is present in the PSA and **hGK-1** promoters, closely resembling the consensus sequence for steroid-responsive elements.

This new gene, which we have named KLK4, is 25 kb downstream of the KLK2 gene and follows a region that includes two other putative KLK-like gene fragments.

The addition of CMV enhancer sequences upstream of a single PSA or **hKLK2** promoter substantially but nonspecifically increased **luciferase** expression in all cell lines tested.

The minimal 628-bp PSA and **hKLK2** promoters showed only low-level expression in either PSA-positive or PSA-negative cells and showed no increase with the addition of androgen.

Binding occurred between bp -539 and -399 and bp -349 and -224 in the PSA and KLK2 promoters respectively, which were shown previously to be necessary for AR-mediated transactivation.

MEASUREMENTS: Pituitary total RNA was subjected to both Northern blot analysis, with KLK1 and KLK2 cDNA probes, and KLK-specific reverse transcriptase -- polymerase chain reaction (RT-PCR).

Using electrophoresis mobility shift assays (EMSA), a common nuclear protein(s) which binds upstream of the androgen-responsive elements (AREs) in the PSA and KLK2 promoters was identified.

The expression of KLK2 and KLK3 in the pituitary is a novel finding.

The KLK1 gene is positioned in the opposite orientation of the APS and KLK2 genes in the order KLK1-APS-KLK2.

KLK2 and KLK3 have important applications in prostate cancer diagnostics and, more recently, in breast cancer diagnostics.

Fine-mapping of the genomic locus indicates that zyme lies upstream of the NES1 gene and downstream from the PSA and KLK2 genes.

top

By RT-PCR, **hKLK2 mRNA** was detected in 7 patients (33%), and hKLK3 **mRNA** was detected in 17 (81%) of 21 stage D **prostate cancer** patients.

3. The similarity in tissue specificity of expression of hGK-1 with that of the PSA gene led to an examination of putative regulatory DNA.

These results suggest that other cis-acting elements may be involved in coordinating in vivo androgenic induction of **hKLK2** and hKLK3 genes.

The significance of the novel expression of KLK2 and KLK3 (PSA), previously thought to be prostate-specific genes, in the endometrium is unclear.

Thus, **antiandrogens** act differentially on androgen-regulated prostate-

specific (PSA, hGK-1) and growth-related (c-myc) gene expression in LNCaP cells.

We conclude that taking advantage of the difference between hKLK2 mRNA and hKLK3 mRNA expression is clinically useful for following up prostate cancer patients.

In addition, multiple AREs from both hKLK2 and hKLK3 were able to reconstitute androgenic induction, further strengthening the argument that the AREs are functional.

We report here the identification of unusual mRNA splice variants of the KLK2 and KLK3 genes that result from inclusion of intronic sequences adjacent to the first exon.

CONCLUSIONS: The repression of AR-mediated induction of PSA and hKLK2 genes by Ca++ mobilizers is due to the interference of AR transactivation activity by AP-1 proteins.

A 1.2 kb cDNA (pGK-1) contains an open reading frame of 510 bp, encoding the major part of the previously predicted hGK-1 protein (Schedlich et al. (1987) DNA 6, 429-437).

In addition, use of sequence-specific primers demonstrated the presence of mRNA for the hGK-1 gene, but not for the hPK gene product or the gene for prostate-specific antigen.

However, placing a 1455-bp PSA enhancer sequence upstream of either the PSA or hKLK2 promoters increased expression 20-fold in the PSA-positive cell line LNCaP but not in the PSA-negative lines.

Marked identity of greater than 90% was seen (compared with 80% for hRKALL), thus supporting a role for the operation of similar tissue-specific mechanisms for the control of hGK-1 and the PSA gene.

We then demonstrated some differences in characteristics, such as differentiation of cancer cells and response to antiandrogen therapy, between hKLK2 and hKLK3 mRNA-expressing prostate cancer cells.

On the basis of information on other genes that are localized in the same region (prostate-specific antigen, KLK2, zyme, and normal epithelial cell specific-1 gene), we speculate that prostase/KLK-L1 may be involved in the pathogenesis and/or progression of prostate, breast, and possibly other malignancies.

The APS and KLK2 gene are separated by 12 kb; the distance between KLK1 and APS is 31 kb.

4. Like KLK2 and KLK3, the KLK5 gene is regulated by steroid hormones in the BT-474 breast cancer cell line.

This review collates in detail current knowledge on the molecular profile and status of TK (hKLK1, hKLK2, and hKLK3) and the kinin B1 and B2 receptor genes.

We have shown by reverse transcription-PCR, using PCR primers directed to the NH2 terminal coding region of the KLK3 (PSA) gene and the closely related KLK1 and KLK2 genes, that these genes are not expressed in these tumors.

Using the known kallikrein or kallikrein-like genes PSA, KLK2, enzyme and normal epithelial cell-specific 1 gene (NES1) as landmarks, we have identified another six novel genes of which, five have protein homologies and gene structure similarities with other kallikreins or kallikrein-like genes.

top

A second cDNA (pGK-10A), with a size of 1.5 kb, contains an open reading frame of 669 nucleotides preceded by 16 nucleotides of the 5'-

untranslated region. pGK-10A differs from pGK-1 by the presence of an additional 37 bp fragment, interrupting the protein coding region of hGK-1, which results from the use of an alternative splice donor site of intron IV of the hGK-1 gene.

Additionally, our study clearly demonstrated that the detection of hKLK2 mRNA in the peripheral blood was useful for screening patients with certain prostate cancers that did not express hK3.

CONCLUSIONS: Gene amplification of hKLK2 may be one of the factors leading to higher expression of hK2 in prostate carcinoma.

Although previous studies have shown that hKLK3 mRNA is expressed at a higher level than that of hKLK2, our results suggest that the hKLK2 ARE may have higher androgenic inducibility than the hKLK3 ARE.

From comparison of the background of the patients positive for hKLK2 and/or hKLK3 mRNA, it became evident that the response to antiandrogen therapy and the expression of hKLK2 mRNA were reciprocally correlated, in contrast with the expression of hKLK3 mRNA.

The authors report that the hK2 gene (hKLK2) was amplified in prostate carcinoma tissue, whereas the hPSA gene was not.

A high level of hGK-1 expression was found in the androgen-dependent tumors PC 82 and PC EW. hGK-1 mRNA was also present in the androgen-sensitive LNCaP cell line, but undetectable in the androgen-insensitive prostate tumors PC 133, PC 135 and the PC 3 cell line.

The expression of 11 nuclear receptor co-regulatory factors (SRC-1, AIB1, ARA24, ARA54, ARA55, ARA70, ARA160, FHL2, PDEF, NCoR1, SMRT) was compared in these cell lines by semi-quantitative RT-PCR to determine if the pattern of receptor co-activators or -repressors expressed in these cells might explain the differential regulation of KLK2 and KLK3.